Supporting Information

Compact and Blinking-Suppressed Quantum Dots for Single-Particle Tracking in Live Cells

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Compositional Analysis. The final product composition of the graded alloy shelled QDs was determined with x-ray photoelectron spectroscopy (XPS) using an Al-Kα source on a powder sample upon a glass coverslip bound with double sided carbon tape. Scans were performed at 160 eV at 0.1 eV resolution with 200 ms dwell times and compositions were obtained from the Zn_{2p3}, Cd_{3d5}, and Se_{3d5} peaks through the Thermo Advantage software. The expected final composition of the QDs from the ratios of reagents put in the reaction is 6.25% Cd, 43.75% Zn, and 50% Se which closely matches to that obtained from the XPS spectrum of the sample (3.51% Cd, 44.22% Zn, and 52.28% Se).

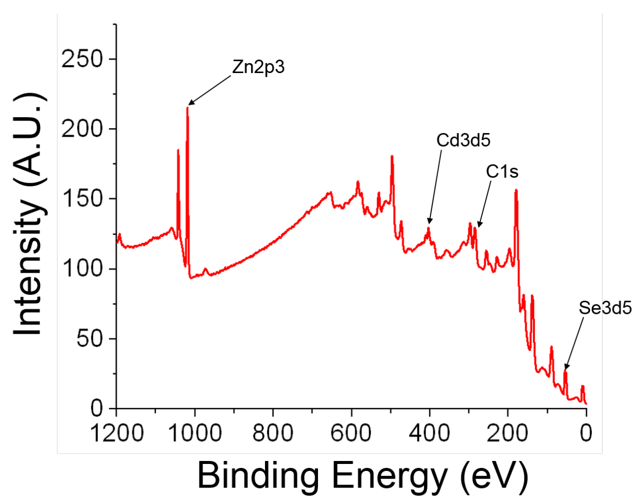

Figure S1. XPS spectrum of the graded alloy shell QDs.

High-Resolution TEM. High resolution TEM of the resulting graded alloy shell QDs was performed by Dr. Haijun Qian using a Hitachi H-9500 operated at 300 kV at the Clemson University Electron Microscopy Facility. The mean particle size of 10.1 nm±1.2 nm was determined by inspecting 200 different particles through the use of the analysis software Image J.

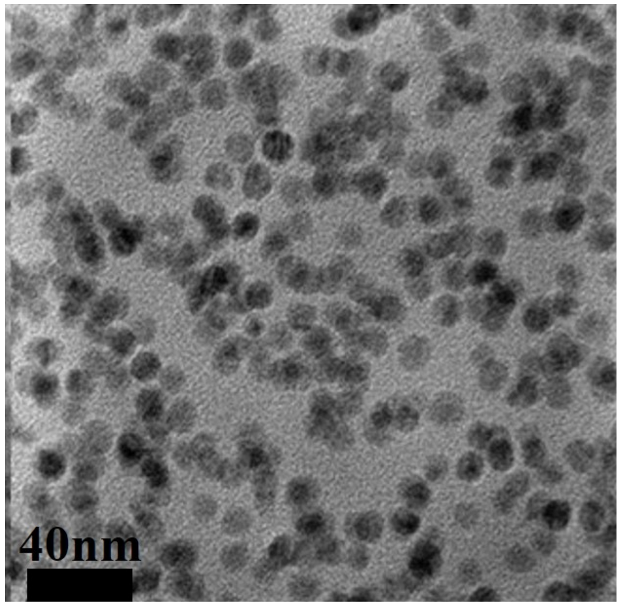

Figure S2. TEM of the CdSe/Cd1-xZnxSe core/shell particles.

Atomic Force Microscopy. Single QDs were verified using an atomic force microscope (AFM, Asylum's MFP-3D-BIO). Dynamic luminescent traces which were obtained from regions which contained particles spaced closer than the diffraction limit were rejected.

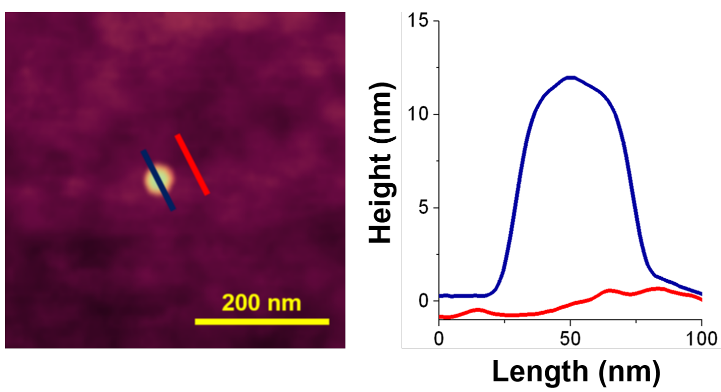

Figure S3. Sample AFM scan showing a single particle in the region where a dynamic luminescent trace was obtained.

Fluorescence Lifetime Measurements. Fluorescent lifetimes of the graded shell QDs and CdSe/ZnS particles were recorded with more concentrated solutions of particles spun onto glass slides then excited with a larger illumination window to record the ensemble. The CdSe/ZnS particles displaying multiexponential behavior (34% 1.8 ns, 56% 10.2 ns, 10% 22 ns), and the characteristic lifetime $\sum P_i t_i^2 / \sum P_i t_i$ was calculated to be 12.6 ns. Similarly, the graded shells with a more monoexponential behavior (11% 2.1 ns, 89% 24.3 ns) results in a characteristic lifetime of 24.0 ns which is only a 1.3% difference from the dominant component. Lifetimes were fit with $\chi^2 \leq 1.2$ using the FAST software from Edinburgh Instruments.

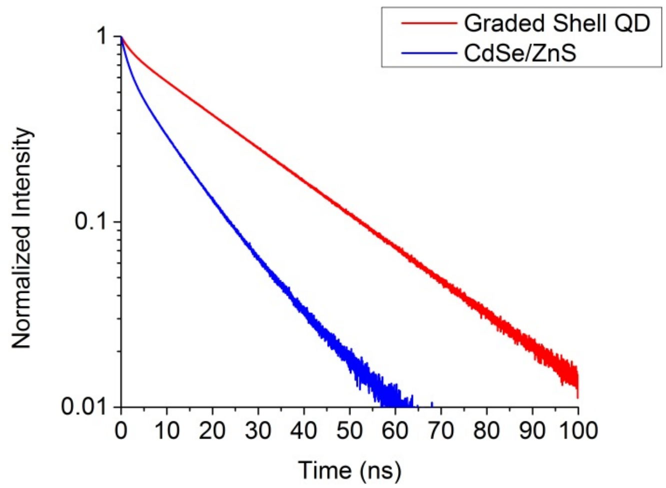

Figure S4. Fluorescent lifetimes of the graded shell QDs compared to cores of similar size with 2.0 monolayers of ZnS (24.3 ns and 10.2 ns respectively

Solubilization and Surface Coatings. The multidentate polymer employed in this work was originally developed by Smith and Nie.[1] The polymer is composed of a polyacrylicacid backbone which is functionalized with thiol and amine anchoring groups through conjugation of cysteamine and ethyldiamine. Thioctic acid conjugated to 750 molecular weight methoxypolyethylene glycol amine (TA-750PEG-OCH_3)[2] is a bidentate molecule presenting two thiol anchoring groups. The monodentate single thiol-anchored PEG of 5000 molecular weight (HS-5000PEG-OCH_3) was used from the supplier (Aldrich) without any further modifications.

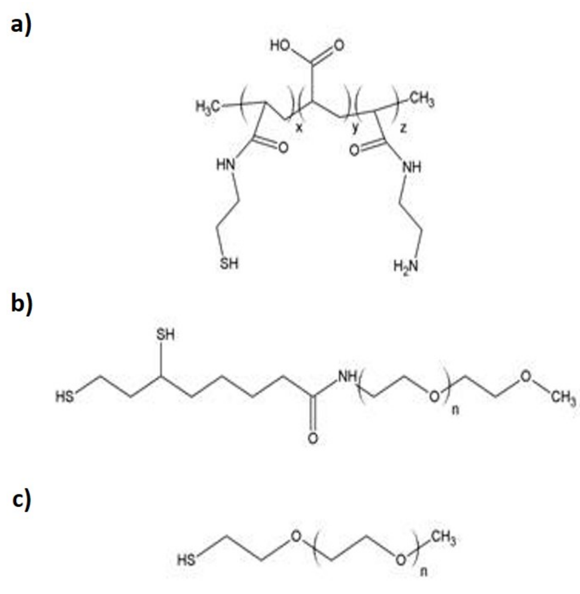

Figure S5. The chemical structures of the ligands employed in aqueous stabilization of the QDs in this study consisting of the multidentate polymer, DHLA conjugated to 750 molecular weight PEG, and 5000 molecular weight thiolated PEG (a, b, and c respectively).

Quantum Yield Measurements. Quantum yields (QY) were obtained by using a standard of fluorometric grade Rhodamine 6G (R6G) dye dissolved in methanol using the following equation:

$$QY_{QD} = QY_{R6G} \frac{I_{QD}}{I_{R6G}} \frac{A_{R6G}}{A_{QD}} \frac{n_{QD}^2}{n_{R6G}^2}$$

here QY_{R6G} is the quantum yield of the standard R6G in methanol having a value of 0.95. I_{QD} and I_{R6G} are the integrated fluorescent spectra from the QD and R6G samples respectively. The spectral intensities of the data are corrected for the changes in quantum efficiency of the detector at different wavelengths. A_{QD} and A_{R6G} are the absorbance of the solutions at the wavelength of excitation of the sample for the fluorescent spectra. In this case it was 450 nm excitation for the QD sample and 525 nm for R6G. n_{QD}^2 and n_{R6G}^2 are the refractive indices squared for the solutions of the QD (hexane=1.375, or water=1.333) and R6G (methanol=1.328) samples respectively.

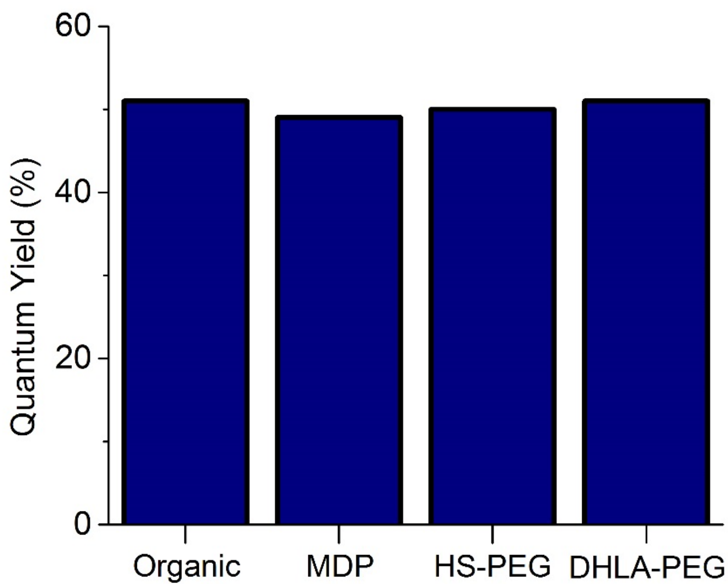

Figure S6. Quantum yields of the gradient alloy shelled QDs coated with the as-synthesized organic ligands solvated in hexanes (Organic), and aqueous solvated particles coated with the multidentate polymer, 5000 molecular weight thiolated PEG, and DHLA conjugated to 750 molecular weight PEG (MDP, HS-PEG, and DHLA-PEG respectively).

References:

1. Smith, A. M.; Nie, S., Minimizing the Hydrodynamic Size of Quantum Dots with Multifunctional Multidentate Polymer Ligands. *Journal of the American Chemical Society* **2008,** *130* (34), 11278-11279.

2. Mei, B. C.; Susumu, K.; Medintz, I. L.; Delehanty, J. B.; Mattoussi, H. *New ligand design to promote water compatibility of luminescent quantum dots and gold nanoparticles*, Proceedings of SPIE, **2008**; pp 686606.1-686606.10.